Antisocial Networking in T Helper Cells

Duy Pham[1] and Mark H. Kaplan[1,*]
[1]Departments of Pediatrics, Microbiology and Immunology, Wells Center for Pediatric Research, Indiana University School of Medicine, 1044 West Walnut St. Indianapolis, IN 46202, USA
*Correspondence: mkaplan2@iupui.edu
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T helper cell differentiation is controlled by a network of transcription factors. In this issue of *Immunity*, Yagi et al. (2010) describe the opposing effects of transcription factors Runx3 and GATA3 in the network promoting T helper 1 cell development.

In a reductionist view of T helper (Th) cell differentiation, cartoons often portray a single "master regulator" transcription factor associated with the development of each subset. In reality, the coordinated activity of multiple transcription factors is required to promote a T helper cell phenotype and to induce the expression of individual genes.

During Th1 cell development, the induction of T-bet, a T-box transcription factor that promotes the expression of many Th1 cell genes, is initially through an interferon-γ-signal transducer and activator of transcription 1 (IFNγ-STAT1)-dependent phase and then through an interleukin-12 (IL-12)-STAT4-dependent phase (Lighvani et al., 2001; Schulz et al., 2009). However, this is not a simple linear pathway. STAT4 and T-bet cooperate in the activation of many Th1 genes, although each can activate a subset of genes expressed in Th1 cells in the absence of the other factor (Thieu et al., 2008). The transcription factor Runx3 is also part of this network, being induced by STAT4 and T-bet and cooperating with T-bet in the induction of *Ifng* and the repression of *Il4* (Djuretic et al., 2007; Naoe et al., 2007; Thieu et al., 2008).

Yagi et al. (2010) provide data from a series of elegant experiments designed to further understand how Runx3 contributes to *Ifng* regulation. Their interest was initially piqued when studying the function of GATA3, the critical transcription factor for Th2 development, using mice with a conditionally deleted *Gata3* allele. They noticed that although GATA3-deficient T cells cultured under Th2 cell-polarizing conditions had decreased Th2 cell cytokine production, they had increased IFN-γ secretion that was not accompanied either by STAT4 activation or induction of T-bet expression. These results suggested that additional transcription factors were involved in the induction of *Ifng* independent of T-bet and STAT4. They demonstrated that Runx3 expression was modestly increased and that expression of Eomesodermin (Eomes), another T-box transcription factor that is associated with *Ifng* regulation in $CD8^+$ T cells, was more dramatically increased in GATA3-deficient Th2 cell cultures. They further showed that Runx3- or Eomes-expressing retroviruses transduced into Th2 cultures were able to promote IFN-γ production. The induction of IFN-γ was not entirely dependent on T-bet because Eomes and Runx3 promoted IFN-γ production in $Tbx21^{-/-}$ cells, and Runx3 was able to induce IFN-γ production in the presence of a dominant-negative T-bet that also blocks Eomes activity. However, it is not clear whether this represents a separate pathway and, whether in GATA3-sufficient cells, there are signals that might induce Runx3 expression independently of T-bet and STAT4.

Yagi et al. (2010) show that retroviral expression of Runx3 induced *Eomes* mRNA, suggesting that a T-bet-Runx3-Eomes pathway is operating in promoting *Ifng* expression, where each factor could have direct effects on the *Ifng* gene (Figure 1). Eomes is detected at high amounts in $Gata3^{-/-}$ Th2 cell cultures, but it is still unknown what would induce it in wild-type cells because Eomes was undetectable by intracellular staining in wild-type Th2 cells or in Th1 cells. Another report, using quantitative PCR, observed increased expression of *Eomes* in $CD4^+$ T cells that lack expression of STAT6, a GATA3-inducing factor (Yang et al., 2008), further supporting potent role for a STAT6-GATA3 pathway in inhibiting *Eomes* expression. Importantly, Yagi et al. (2010) demonstrated the important role of Runx3 in Eomes activity by showing that Th2 cultures of cells that are triply deficient in GATA3, T-bet, and Runx3 had diminished IFN-γ production compared to cells deficient only in GATA3 and T-bet (approximately half of the Eomes-positive cells in double-deficient cells were IFN-γ positive versus only 17% in triple-deficient cultures). These results suggest that whereas Eomes can induce IFN-γ production, activity is not optimal in the absence of other factors and Runx3 is required for the STAT4-T-bet-independent regulation of IFN-γ. Answering the question of whether limited expression of Eomes, that is undetectable by intracellular staining, is important for IFN-γ production awaits the generation of *Eomes* conditional mutant mice.

The authors go on to further characterize the ability of Runx3 to contribute to *Ifng* expression. They demonstrated that a dominant-negative Runx3 reduced IFN-γ production from WT Th1 cells and that CBF-β, the binding partner of multiple Runx family members, bound to the *Ifng* gene in a pattern that largely overlaps with the binding pattern of T-bet. This is consistent with the previous report suggesting the cooperative binding of Runx3 and T-bet to the *Ifng* promoter (Djuretic et al., 2007). It will be very important to see further analysis of the T-bet and Runx3 ChIP-seq data sets produced by Yagi et al. (2010) to develop a more complete understanding of how these factors interact in programming Th1 cell gene expression.

As suggested by the initial observations, the ability of Runx3 to activate the *Ifng* gene is dependent on the amount of GATA3 expression. Using retroviral transduction and analysis of cells across a range of expression of both Runx3 and GATA3, they demonstrated that the

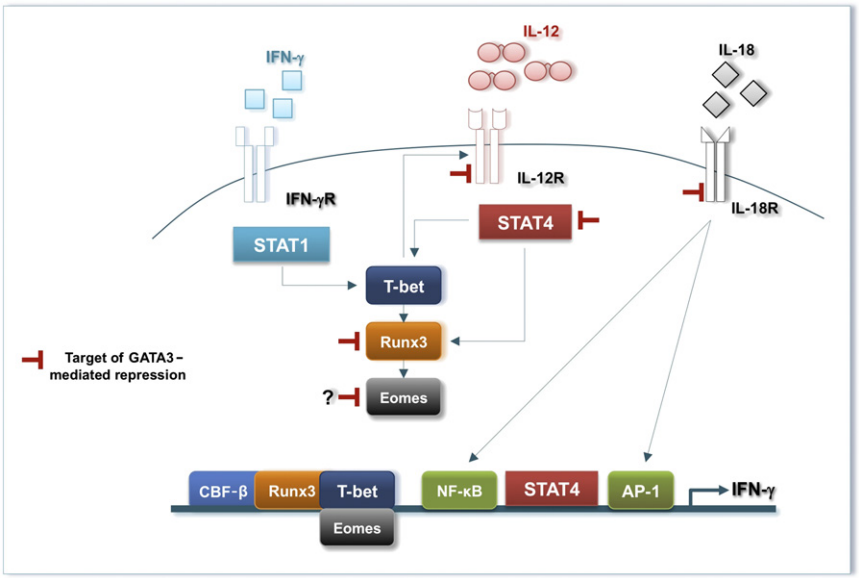

Figure 1. Transcriptional Network in Th1 Cells

Cytokine signaling pathways activate transcription factors that have direct effects on the *Ifng* gene. STAT1 and STAT4 initiate a cascade of transcription factor induction through regulation of T-bet and other downstream factors, each of which can also bind to, and activate, the *Ifng* gene. Targets of GATA3-mediated repression are indicated by red flat arrows. Yagi et al. (2010) have added to this network by demonstrating that Runx3 is a target of GATA3 and that in the absence of GATA3, Runx3 and Eomes mediate *Ifng* induction in the absence of the upstream factors STAT4 and T-bet.

repression of IL-4 by Runx3 is largely independent of GATA3. In contrast, the ability of Runx3 to stimulate IFN-γ production was severely limited by GATA3, and in cells expressing high amounts of GATA3, even high expression of Runx3 was unable to increase IFN-γ production. The basis for this is a physical interaction between Runx3 and portions of GATA3 that include the N-terminal region and the zinc finger. These observations are confirmed by another report that demonstrates the ability of Runx3 to induce IFN-γ production and interact with GATA3 (Kohu et al., 2009).

An additional insight into the negative-regulation of *Il4* is provided by experiments that compare Runx3-mediated repression of *Il4* in wild-type and *Tbx21*$^{-/-}$ Th2 cells. A previous report suggested that T-bet and Runx3 cooperate in the repression of *Il4* by binding to the *Il4* HSIV element, and demonstrated that T-bet-mediated repression of *Il4* required Runx3 (Djuretic et al., 2007). Yagi et al. (2010) demonstrated that Runx3 is capable of repressing IL-4 production in T-bet-deficient cells. This suggests that T-bet-dependent repression of *Il4* may be through the ability of T-bet to inhibit GATA3, and to induce Runx3 expression, and not due to direct effects of T-bet on the *Il4* locus. In support of this, supplemental data of ChIP-seq analysis indicates that CBF-β but not T-bet bound to the *Il4* HSIV element.

The characterization of Runx3 as a GATA3-interacting protein adds further complexity to our understanding of how GATA3 interferes with Th1 cell-inducing signaling pathways. Previous work has demonstrated that GATA3 interferes with the expression of IL-12Rβ2 and IL-18Rα, as well as STAT4, which is thought to be a critical factor in maintaining the ability of cells to acquire IFN-γ-secreting potential (Usui et al., 2003; Yu et al., 2008) (Figure 1). It is still not clear if GATA3 regulates *Eomes* directly, though the increased expression of *Eomes* in Th cells that lack STAT6 or GATA3 (Yang et al., 2008; Yagi et al., 2010) supports the ability of the IL-4 signaling pathway to potently decrease *Eomes* expression. The direct interaction of Runx3 with GATA3 facilitates another sensitive molecular switch in developing Th cells. GATA3 not only prevents Runx3 from activating IFN-γ and potentially other Th1 genes but also inhibits it from repressing *Il4*. Thus, the balance in the development of Th1 and Th2 cells is controlled by the direct interactions of these two transcription factors.

One important caveat in interpreting these experiments is realizing that Th2 cells transduced with Runx3 or Eomes do not become Th1 cells, despite the induction of IFN-γ. The authors show that Eomes is less efficient than T-bet in the induction of *Ifng* and *Il12rb2*, although they had similar abilities to induce *Cxcr3*. This distinction is also seen when comparing the intracellular staining for IFN-γ in Th1 cells and transcription factor-transduced Th2 cells, or *Gata3*$^{-/-}$ Th2 cells, considering both the percentage of positive cells and the fluorescence intensity of the cells that are positive. The ability of any one factor to promote only a partial phenotype in the absence of other factors again highlights the requirements for a network of transcription factors in establishing a complete Th cell phenotype. This network, summarized in Figure 1 to indicate the multiple factors that promote *Ifng* expression, as well as those that are targets of GATA3-mediated repression, provides a developing framework for understanding how these and other transcription factors cooperate in programming gene expression and cell-fate determination.

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